Validating the genetic alterations in cutaneous T-cell lymphoma: unraveling the role of SOCS1 and HNRNPK through genetically engineered mouse models

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General introduction

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This chapter was adapted from “Next top mouse models advancing CTCL research”

“Next top” mouse models advancing CTCL research

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Introduction

Cutaneous T-cell lymphoma (CTCL), a rare form of non-Hodgkin lymphoma, accounts for approximately 3% of all lymphoma cases and presents unique challenges in oncological research. Characterized by malignant T-cell accumulation in the skin, often without initial spread beyond this organ, CTCL exemplifies the complexity and variability of rare malignancies (1). CTCL represents a heterogeneous group of disorders, including subtypes such as mycosis fungoides (MF), Sézary syndrome (SS) and CD30+ lymphoproliferative disorders (LPDs). While primarily a skin disease, CTCL can evolve into systemic lymphoma, spreading to lymph nodes and internal organs. As it constitutes approximately 75% of all primary cutaneous lymphomas, understanding CTCL's intricate pathobiology demands comprehensive and detailed research approaches (2). In this regard, *in vivo* mouse models are potentially powerful tools in unraveling the complexities of CTCL's pathogenesis. Such models can lead to the design of well-targeted early-stage treatments, which can then be preclinically tested in these experimentally accessible models.

In this introductory chapter, we thoroughly examine various *in vivo* mouse models currently at the forefront of CTCL research. This includes a focused discussion on the latest developments in transplantation models and genetically engineered mouse models (GEMMs). We will clarify the subtypes of CTCL if the references classify the model clearly. When the model's CTCL subtype is not clear, we will use the term 'CTCL model' broadly to encompass the diverse spectrum of this disease. Each model provides insights into different aspects of the disease, from tumor-host environment interactions and gene functions to drug efficacy validation. These contribute to our deepening understanding of CTCL and aid in the advancement of innovative therapeutic approaches. Here we will categorize and introduce mouse models of CTCL including the next top mouse models, serving as a reference for researchers unfamiliar with mouse experimentation when selecting models for CTCL research.

1. Transplantation Mouse Models in CTCL Research

Transplant models are essential in CTCL research and typically involve transplanting (human) donor cells or tissues into a recipient organism (mice). CTCL transplant models focused on late-stage human cutaneous lymphomas. These 'Xenograft models' involve transplants between different species, requiring immunodeficient mice to receive human CTCL cells or tissues. However, these models lack a fully competent immune system, which is a significant limitation, as it prevents a complete understanding of immune system interactions in CTCL. Additionally, they primarily focus on established tumors, offering limited insight into the early stages of CTCL pathogenesis. This underscores the need

for cautious interpretation of results from these models, especially regarding immune response and early disease development. 'Syngeneic transplant models' use genetically identical mice to avoid graft-host reactions and preserve normal interactions between tumor and immune system, but do not involve genuine human CTCL. These models, detailed in subsequent sections, offer valuable insights into CTCL pathogenesis and treatment response (3).

1.1. Immunodeficient Mouse Models with Transplantation

Immunodeficient mouse models are critical for CTCL research, allowing the study of tumor progression and response to treatments. These models are particularly valuable for precision medicine, enabling individualized testing of medication in the laboratory to circumvent disease heterogeneity. Patient-derived xenograft (PDX) and cell line-derived xenograft (CDX) models, wherein tumor cells from patients or established cell lines from MF, SS and other CTCL subtypes are transplanted into immunodeficient mice, play a key role. Among the cell lines utilized to study CTCL, SeAx, Sez4, SZ4, H9, and Hut78 correspond to SS origins, providing insight into this subtype. Similarly, Myla and HH cell lines reflect advanced MF, while Mac2A and PB2B are indicative of CD30+ LPDs, and MJ and Hut102 lines are associated with Adult T-cell Leukemia/Lymphoma (ATLL), demonstrating the broad spectrum of CTCL manifestations (4) . The recipient mice for the PDX and CDX model, due to targeted genetic modifications that eliminate certain crucial immune functions, do not reject the transplanted cells or samples (5). However, the absence of a fully functional tumor microenvironment and a comprehensive host immune response are notable limitations of these models.

Current CTCL research lacks comprehensive studies comparing engraftment efficiency and metastatic rates in various immunodeficient mouse strains (6-8). Predominantly, NSG, NOG, and NRG mice have been preferred in recent CTCL studies due to their superior engraftment capabilities, particularly effective in researching human acute leukemia and melanoma (9, 10). Under specific pathogen-free conditions, these strains exhibit longer lifespans, enhancing their value in xenotransplantation. The pioneering nude mouse model, despite its historical significance in cancer research, shows lower engraftment success (6).

Other strains like NSB, C.B-17 SCID Beige, and *Rag2 -/- γ -/-* mice, despite shorter lifespans, display impressive engraftment abilities. Each strain offers unique traits that are beneficial for specific research purposes. For instance, NOD SCID mice are crucial in studying the pruritic phenotype of CTCL (11, 12).

Selecting the right immunodeficient mouse strain is critical for CTCL research and depends on study goals, graft nature, and experimental conditions. Thoughtful selection is key to translating preclinical results into clinical applications and advancing CTCL understanding and treatment. Below we outline and compare various immunodeficient mice used in CTCL research (refer to **Tables 1 & 2**).

Table 1. The main features of immunodeficient mouse models for transplantation in CTCL research.

Mouse Strain	Mutated gene	Cell population change	Main Features
NSG (6)	*Prkdc*^{scid} *Il2rg*^{tm1Wjl}	No T cells No B cells No NK cells	Long lifespan: a median survival time of 89 weeks. High engraftment ability.
NOG (7)	*Prkdc*^{scid} *Il2rg*^{tm1Sug}	No T cells No B cells No NK cells	Similar to NSG Long lifespan. High engraftment ability.
NRG (13)	*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}	No T cells No B cells No NK cells	Similar to NSG Long lifespan. High engraftment ability.
NSB (9)	*Prkdc*^{scid} *B2m*$^{tm1Unc/J}$	No functional T cells No functional B cells Diminished NK cells No MHC class I No complement factor C5	Short lifespan: the average lifespan 30 weeks. High engraftment ability

Nude nu/nu (14)	*Foxn1*^{nu}	No T cells	Moderate lifespan: 6 months to one year. Low engraftment ability.
NOD SCID (11)	*Prkdc*^{scid} (NOD background)	No functional T cells No functional B cells	Short lifespan: the median survival time: 37 weeks Moderate engraftment ability. Slightly lower than NSG, NOG and NRG
CB 17 SCID (15)	*Prkdc*^{scid} (C.B-17 background)	No T cells No B cells	Moderate lifespan: around one year. Moderate engraftment ability.
CB17 SCID beige (16)	*SCID* beige	No T cells No B cells Defective NK cells	Moderate lifespan High engraftment ability.
Rag2 -/- γc-/- (17)	*Rag2*$^{tm1.1Flv}$ *Il2rg*$^{tm1.1Flv}$	No T cells No B cells No NK cells	Short lifespan: the average lifespan 34 weeks. High engraftment ability.

Table 2. Transplantation methods for immunodeficient mouse models in CTCL research

Mouse Strain	Subcutaneous injection	Intrahepatreal injection	Intrafemoral injection	Intravenous injection	Skin/tumor grafting
NSG (6)	- SS Blood-derived PBMCs: 1× 10^6 (18) - MF lymph node-derived leukocytes: (5-10) × 10^6 cells (19) - PDX mouse tumor-derived: 4 × 10^6 cells (20) - CTCL cell lines: Myla (MF cell line): 0.8 ×10^6 cells, transferred with miR-125b-5p (21) Hut78 (SS cell line) or HH (MF cell line): 1× 10^6 cells, with TOX knockdown with shRNA (22) Hut78 (SS cell line) or Myla (MF cell line): 2.5 × 10^6 cells (23) Hut78 (SS cell line) or HH (MF cell line) : 1× 10^6 cells (24) HH (MF cell line): 2 ×10^6 cells (25) Myla (MF cell line), PB2B (CD30+ LPDs cell line), HH (MF cell line), H9 (SS cell line), Hut78 (SS cell line), SZ4 (SS cell line), MJ (ATLL cell line), or Hut102 (ATLL cell line): 2.5 × 10^6 cells (26) Hut78 (SS cell line), H9 (SS cell line), MJ (ATLL cell line), or HH (MF cell line): 10 × 10^6 cells (19)	-CTCL cell lines: Myla (MF cell line), Hut78 (SS cell line), HH (MF cell line): 5 ×10^6 cells (32)	-SS Blood-derived PBMCs: 1× 10^6 cells (18)	-SS Blood-derived PBMCs: (5 ~ 20) × 10^6 cells (33) -CTCL cell lines: Hut102 (ATLL cell line): 10 ×10^6 cells (34) (35) HH cells were transduced with lentivirus to express click beetle luciferase green and green fluorescent protein and were further transduced to express runcated CD19 (HH-CBG-GFP-t19) (36) HH (MF cell line) (HH-CBR-GFP): 0.4× 10^6 cells (37)	-

	Hut78 (SS cell line) or HH (MF cell line): 5 × 10^6 cells (27) (28) SeAx (SS cell line): 0.4 × 10^6 cells (ear) (29) SeAx (SS cell line): 3 × 10^6 cells (flank) (30) MJ (ATLL cell line): 3.8 * 10^6 cells /sites, transduced with INSL3-shRNA-TRC58, -TRC61: 3.8× 10^6 cells /sites (31)				
NOG (7)	- CTCL cell lines: HH (MF cell line): 10 × 10^6 cells (38) Myla (MF cell line): 0.2 × 10^6 cells ; HH (MF cell line): 2 × 10^6 cells; Hut78 (SS cell line): 4 × 10^6 cells (39) Myla (MF cell line): 1× 10^6 cells (40) Myla (MF cell line) or HH (MF cell line): 0.2 × 10^6 cells, transfected with GFP-miR-150 (41, 42)	-	-	-	-
NRG (13)	- CTCL cell lines: Luciferized Hut78 (SS cell line): 2× 10^6 cells (8)	-	-	-CTCL cell lines: CD38 knockout luciferized H9 (43)	-
NSB (9)	- CTCL cell lines: Myla2059 (MF cell line): 1× 10^6 cells (44) (45) Myla2000 (MF cell line): 1× 10^6 cells (46)	-	-	-	-
Nude nu/nu (14)	- CTCL cell lines: Myla (MF cell line): 10× 10^6 cells (47) HH (MF cell line): 20 × 10^6 cells (48) HH (MF cell line): 10× 10^6 cells (49) (50) HH (MF cell line): 2 × 10^6 cells (51)	-	-	-	- Myla-derived mouse tumor fragments: 5×3 mm subcutaneous (47)

NOD SCID (11)	- CTCL cell lines: Myla (MF cell line): 10× 10^6 cells (52) (53) Hut102(ATLL cell line): 2 ×10^6 cells (54) HH (MF cell line): 20 × 10^6 cells, transduced with the PAK1-sh2-vector (55) HH (MF cell line): 5 × 10^6 cells, transduced with BIN1 - siRN (56) SeAx (SS cell line): 0.2 × 10^6 cells (57)	-	-	- CTCL cell lines: Hut102(ATLL cell line): 10× 10^6 cells, with sublethally irradiated (1.8 Gy) (54)	
CB 17 SCID (15)	- CTCL cell lines: HH (MF cell line): 5× 10^6 cells (58)				- Skin grafting from SS: 4 × 7 × 0.75mm into 4 pieces (15)
CB17 SCID beige (16)	- CTCL cell lines: Hut78(SS cell line) or Myla 2059 (MF cell line): 3 × 10^6 cells (59) HH (MF cell line): 1 × 10^6 cells (60)	-	-	-	-
Rag2 -/- γc-/- (17)	-	- CTCL cell lines: Hut78 (SS cell line) or SeAx (SS cell line): (0.5 - 6) ×10^6 cells (61)	-	- SS Blood-derived PBMCs: (0.5 -1) × 10^6 cells (with irradiation) (62)	

CTCL: cutaneous T cell lymphoma; PBMC: peripheral blood mono-nuclear cell; SS: Sézary syndrome; MF: mycosis fungoides

1.1.1. NSG mouse in CTCL

NSG mouse strain, formally named NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}, is indispensable for CTCL studies because of its broad immunodeficiency (6). Their unique genetic background amalgamates traits from NOD, SCID(*Prkdc*^{scid}), and gamma mutation (*Il2rg*^{tm1Wjl}), resulting in the absence of functional T cells, B cells, and NK cells. The profound immunodeficiency of NSG mice positions them as an exemplary recipient for development of intrahepatic xenograft models of CTCL, facilitating the evaluation of tumorigenicity and therapeutic responses. The maintenance of NSG mice requires stringent pathogen-free conditions due to their lack of immune defenses, which has implications for the management and costs of these studies. Despite this, the NSG model's inability to mount an adaptive immune response offers an excellent recipient.

With the aid of this model, researchers have progressively unveiled tumor-driving pathways and corresponding treatment of CTCL, e.g. the cMyc/miR-125b-5p signaling axis (21), *TOX* genes (22), Mucin 1 (23), and the potential therapeutic effects of gallium maltolate in inhibiting tumor growth (24). Furthermore, the NSG mouse model has demonstrated its utility in the rapid assessment of CTCL (32), and in subsequently testing novel therapeutic modalities, in particular employing demethylating agents in conjunction with mucin 1 inhibitors (25). These studies not only underscore the importance of CTCL heterogeneity but also highlight the therapeutic potential of coordinated treatments involving PI3Kalpha/delta and HDAC (18, 19, 26), kinase inhibitors with TAK1 (27, 29), the synergistic combination of Bcl-2 and NFκB inhibitors (30) and the effectiveness of a bispecific IL2-CCR4 immunotoxin (34). Recent advancements include the superior performance of CCR4-IL2 immunotoxin (35), RT39 peptide therapy (33), novel drug NT1721 (28), JAK3-INSL3 fusion transcripts (31), anti-CCR4 CAR T cells (36), universal CD2 CAR-T therapy (37) and the antibody-drug conjugate SGN-CD70A (20) have further expanded the therapeutic research landscape for CTCL.

1.1.2. NOG mouse in CTCL

The NOG mouse model, formally designated as NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}, stands out in CTCL research for its pronounced immunodeficiency, miming severe combined immunodeficiency (SCID) in humans (63). IN close similarity to NSG, this strain is void of functional B and T lymphocytes due to the *Prkdc*^{scid} mutation and lacks natural killer (NK) cells due to the *IL-2Rγ*^{null} mutation, making them an ideal platform for human cell engraftment (38). In researching the effects of microRNAs on CTCL, the NOG model has revealed the tumor-suppressive role of microRNA-16, while IL-22 may facilitate tumor

metastasis (39, 40). Furthermore, miR-150 has demonstrated potential in inhibiting tumor metastasis (41) and histone deacetylase inhibitors targeting miR-150 and CCR6, such as Vorinostat, have presented new strategies for the treatment of advanced CTCL (42).

1.1.3. NRG mouse in CTCL

The official name for the NRG mouse model is NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}. Due to the knockout of the *Rag1* and *Il2rg* genes, this mouse model lacks mature T, B, and NK cells (13).

The studies using NRG mice for CTCL research found that the combined use of chlorpromazine and romidepsin displayed significant antitumor activity (8), and the expression of CD38 is associated with the progression of CTCL, suggesting that CD38 may play a significant role in the immunopathogenesis of CTCL and could potentially become a new target for therapeutic intervention (43).

1.1.4. NSB mouse in CTCL

The NSB mouse model with the official name NOD.Cg-*Prkdc*^{scid}*B2m*$^{tmUnc/J}$, distinct in their immunodeficiency due to a *B2m*^{tm1Unc} mutation affecting MHC class I expression, lack CD8+ T cells and exhibit impaired NK cell function (64). This characteristic enables the strain to support the engraftment of malignant T cells such as Myla2059, providing a robust model for the study of late-stage CTCL, especially MF, dissemination and treatment (44). Additionally, the secretion of molecules such as galectin-1 and -3 by malignant T cells has been associated with the disruption of skin architecture and the proliferation of keratinocytes in CTCL (45, 46).

Distinct from the NOG and NSG strains, which suffer from impaired NK cell function due to mutations in the *IL2R* gamma chain, the deficit in this strain arises from the impact of the *B2m* mutation on MHC class I expression, marking its unique role in the study of CTCL models

1.1.5. Nude (nu/nu) mouse in CTCL

The “nude” (nu/nu) mouse model, which lacks a mature thymus due to a *Foxn1* gene mutation, resulting in underdeveloped T cells (14), has become a critical model for evaluating CTCL therapies, especially in terms of treatment responses for MF patient-derived skin lesions. These mice with transplants of MF have shown enhanced effects of combination therapies, like PUVA and mogamulizumab, a monoclonal antibody targeting CCR4, compared to monotherapies (47, 48). Further studies using “nude” mice have been conducted to test the effectiveness of Vorinostat and the HIF-1α inhibitor Echinomycin,

unveiling their potential in combating CTCL (49, 65). The dual PI3K/mTOR inhibitor PF-502 has also been shown to prolong survival in “nude” mice, suggesting its potential as a promising therapeutic for CTCL (50). And metabolic analysis of CTCL model mice has led to the discovery of fluctuations in L-glutamate and adenosine monophosphate levels, which could contribute to understanding CTCL the dynamics of biomarkers (51).

1.1.6. NOD SCID mouse in CTCL

The NOD SCID mouse model, formally designated as the NOD. CB17-*Prkdc*^{scid} strain, is valuable in CTCL research as recipient mice due to its lack of mature T and B cells, making it suitable not only for studying pruritus—a hallmark symptom of CTCL (12) —but also for investigating tumor growth, early symptoms, and the role of gender in disease mechanisms (52, 53). Employing the NOD SCID mice for the CTCL model, LW-213 has shown notable therapeutic potential, inhibiting the growth of CTCL-associated xenograft tumors and improving survival rates (54). Additionally, this model has been used to validate the role of PAK1 in CTCL cell proliferation and the therapeutic potential of its inhibitors (55), as well as to study the role of BIN1 in disease progression through its regulation of c-FLIP affected Fas/FasL-mediated apoptosis (56). Moreover, the combined application of retinoic acid and histone deacetylase inhibitors has demonstrated antitumor effects (57).

1.1.7. CB17 SCID mouse in CTCL

The CB17 SCID mouse model, originating from the C.B-17 strain, bears a *Prkdc*^{SCID} gene mutation that results in a profound deficiency in adaptive immunity by impairing T and B lymphocytes (66). This strain, as recipient mouse of CTCL from SS patient-derived skin, provides valuable insights into the pathology and can aid in developing new therapeutic strategies (15). Recent research has demonstrated that the combined use of Brentuximab Vedotin (BV) with doxorubicin exhibits significant tumor suppression in the HH cell tumor model in CB17SCID mice, further confirming the potential of this drug combination in the treatment of T-cell lymphomas (58).

1.1.8. CB17 SCID Beige mouse in CTCL

The CB-17 SCID beige mouse model, due to the combined *SCID* and *beige* mutations, possesses an extensive range of immunodeficiencies, including the absence of T cells, B cells, and compromised NK cell function, providing a more comprehensive immunodeficient model than the CB17 SCID defect only (16). As a recipient, these mice excel in tumor studies due to their increased tumor growth rates compared to less immunodeficient nude mice, ideal for aggressive tumor research contrasting slower-progressing SS tumors (59). In CTCL research, these mice, together with the EL4 mouse T-cell

lymphoma model, have aided in discovering that the expression of galectin-9 on tumor cells is inversely proportional to CD8+ T cell infiltration in the skins of EL4 mouse model and serum levels of galectin-9 correlate with disease severity. However, the anti-tumor effect of exogenous high-dose galectin-9 administration demonstrated anti-tumor effects in CTCL, underscore its significance as a potential therapeutic target (60).

1.1.9. Rag2 -/- mouse in CTCL

The Rag2-/- mouse model carries a mutation that disables the *Rag2* gene, essential for T and B lymphocyte development through V(D)J recombination. This mutation results in a complete absence of mature T and B cells, creating a foundational model for immunodeficiency studies (67). While not a primary model for CTCL itself, Rag2-/- mice serve as recipients in specific studies, such as those involving subcutaneous injections of modified CD4+ T cells from Myc+ Cdkn2a -/- mice, to explore the mechanisms of cutaneous hypersensitivity and the immunological roles of IL-7 and IL-15 (68). An enhanced version of this model, the Rag 2-/- γc-/- mice, lack functional T, B, and NK cells due to the knockout of both the Rag2 and the interleukin-2 receptor gamma chain gene (Il2rg or γc), which affects cytokine receptor production. This strain is suitable for xenotransplantation studies on SS, in particular it sustains long-term systemic repopulation with injected SS cell lines or primary cells without immune rejection (61).

The SRG15 mouse, an advanced version of the Rag 2-/- model, combines *Rag2-/- , γc-/-* mutations and humanized IL15 and human signal regulatory protein alpha (SIRPA) mutations (69). These 'humanized' mice are engineered to express human IL-15, accommodating the growth of SS tissue samples more effectively than traditional immunodeficient models. Integrating of human *IL-15* and *SIRPα* genes in the SRG15 mice enables them to support human NK and T cells, making them excellent tools for studying human immune cell behaviors (62).

1.2. Non-immunodeficient Mouse Models with Syngeneic Transplantation

Syngeneic transplantation models, wherein syngeneic lymphomas are introduced into the skin of mice, serve as valuable tools for investigating tumor behavior and host-tumor interactions. These models allow for studying tumor dynamics within a genetically consistent background, offering insights into the tumor's interaction with a native immune system. However, it is crucial to acknowledge that these models have limitations in representing the human immune system and the diverse variants of the disease. Specifically, they lack the complexity and heterogeneity inherent in human CTCLs. Such differences are crucial for researchers to consider, ensuring that the distinct differences

between model and human disease are accounted for in research conclusions and clinical applications.

1.2.1. MBL2 mouse model in CTCL

The Mannose-Binding Lectin 2 (MBL2) mouse model, utilizing C57BL/6 mice as a syngeneic platform, creates an auto transplantation model for CTCL by injecting MBL2 lymphoma cells and inducing inflammation with DNFB. Although not based on genuine CTCLs, this model effectively simulates the impact of inflammation observed in CTCL and highlights the potential of anti-inflammatory treatments such as the PARP-1 inhibitor talazoparib and IL-10 suppression in controlling tumor growth (70-72). Further research has confirmed the efficacy of CD47 blockade agents and CCR2 inhibitors in slowing tumor growth and modulating the tumor microenvironment (73, 74). The discovery that rapamycin inhibits tumor growth by highlighting its impact on the metabolism of lymphoma cells, particularly reducing the reliance on aerobic glycolysis, offers a new avenue for metabolic intervention in treating CTCL (75).

1.2.2. EL4 mouse T-cell lymphoma model

The EL4 mouse model uses a T-cell lymphoma cell line derived from C57BL/6 mice and serves as a syngeneic transplant model for CTCL by virtue of inoculation in the skin with an impact on matching immune system. Studies utilizing this model in CTCL-related research have shown that bexarotene demonstrates immunomodulatory potential by reducing levels of CCL22 (76). Moreover, combining mogamulizumab with PUVA therapy shows enhanced therapeutic effects (77). This model has also demonstrated a possible role for CXCL11 in anti-CTCL treatment (78), revealed the role of TSLP in promoting a Th2-dominant tumor environment (79), and identified galectin-9 as a potential new therapeutic target for CTCL (80). Additionally, the EL4 model has elucidated the role of IL9 and its regulatory factors in MF (80), as well as the importance of PlGF in promoting lymphoma cell growth and disease progression in CTCL (81).

1.2.3. Murine bone marrow transplantation model

The bone marrow transplantation model for CTCL, examining the *JAK3*^{A572V} mutation, provides insights into lymphocyte development and the mutation's role in T-cell proliferation and survival. This model reflects the pathological traits of aggressive lymphoproliferative disorders, including CTCL, with manifestations such as skin involvement in human CTCL. Findings indicate the *JAK3*^{A572V} mutation's capacity to induce a transplantable, diverse CTCL-like disease, exacerbated by trisomy 21, which may result in fatal leukemia from CTCL phenotypes (82, 83). Bone marrow transplantation models

are relatively complex to operate and require high-standard experimental equipment and environments, which limits the application of the model.

2. Non-Skin Target Genetically Engineered Mouse Models (GEMMs) in CTCL Research: carcinogenesis from a to z.

Genetically Engineered Mouse Models (GEMMs) offer a physiologically relevant platform to study human CTCL by introducing specific gene modifications (84). Genomic analysis has identified a number of genes as potential therapeutic targets in CTCL (85). Given that mice share about 85% genetic similarity with humans (86, 87), GEMMs facilitate understanding the role of specific genetic modifications in CTCL development. These models enable the study of natural cancer progression and interaction with the immune system from the onset (88). However, non-skin target GEMMs primarily simulate systemic CTCL pathogenesis and often do not originate from skin-homing CD4+ T cells, the main origin of CTCL genesis, limiting their applicability to skin-centric CTCL features.

2.1. Knockout Mouse Models in CTCL Research: Starting from systemic tumorigenesis.

Knockout mouse models are prevalent in CTCL research, enabling the study of gene function by gene deletion, particularly in the core cell type implicated in CTCL, CD4+ T cells (89). The CD4*CreER*^{T2} transgenic mouse model exemplifies this, where Cre is controlled by the CD4 promoter and gene editing thus selectively targets CD4+ T cells. The Cre/lox system used here allows for temporal and cell-specific gene inactivation via tamoxifen-activated *CreER*^{T2} recombinase. Such inducible knockouts are tools for dissecting gene roles in CD4+ T cells, providing insights into their complex functions in immunity and disease progression. Although, they may predominantly manifest skin symptoms similar to CTCL, they originate from systemic T cell disorders, aligning more with secondary CTCL types (90). It highlights the need for careful consideration when extrapolating findings from these models to primary CTCL.

2.1.1. *R26STAT3C*$^{stopfl/+}$ *CD4Cre* Mouse Model

The *R26STAT3C*$^{stopfl/+}$ *CD4Cre* mouse model is utilized to assess the consequences of persistently active STAT3 in CD4+ T cells. These mice are genetically engineered to have a modified *STAT3C* gene at the *ROSA26* locus (91), which is continuously expressed in CD4 cells due to removing a stop sequence flanked by loxP sites through the CreLoxP system. This persistent activation of STAT3 simulates skin abnormalities akin to those seen in CTCL. Research by Fanok et al. using this model revealed that dysregulated cytokine signaling, particularly aberrations in the IL-2 receptor signaling pathway and the JAK-STAT signaling

pathway, as well as imbalances in microenvironmental factors, like the skin microbiome, may promote the onset and progression of CTCL (92).

2.1.2. *CD4CreER^{T2}Satb1$^{fl/fl}$ Rosa26$^{N1\text{-}ICD}$* Mouse Model

The *CD4CreER^{T2}Satb1$^{fl/fl}$Rosa26$^{N1\text{-}ID}$* mouse model is designed to study the role of SATB1 protein by deletion and Notch1 by overexpression (intracellular domain N1-ICD) in CD4+ T cells (not only those residing in the skin). This model mirrors advanced - CTCL pathogenesis. *SATB1* loss leads to increased chemokine receptors including CCR4, affecting T-cell migration with the transformation of CD8+ T cells into CD4+ CD8+ double-positive T cells and more infiltration of CD3+ T cells in the skin of the mice, and CTCL progression. Moreover, with exhibiting CD8 and CD11b co-expression and symptoms like splenomegaly and lymphadenopathy, it is a valuable tool for exploring late-stage CTCL's advancement and treatment (93).

2.2. Transgenic Mouse Models in CTCL Research

Transgenic mouse models are created by inserting exogenous DNA into the mouse genome, which allows for precise manipulation of gene expression to assess gene function and its impact during a disease (94). In CTCL research, these models are crucial for exploring genes associated with the disease, providing a window on the mechanisms of CTCL onset and progression.

2.2.1. IL-15 Overexpression Mouse Model

The IL-15 overexpression mouse model uses transgenic technology to introduce an exogenous *IL-15* gene into the mouse genome, leading to its overexpression and causing the mice to develop a CTCL-like disease similar to the human condition. IL-15 is a cytokine involved in the maturation of lymphocytes (95, 96). This model mirrors the high levels of IL-15 found in CTCL patients and allows for the observation of clinical symptoms and disease progression *in vivo*, aiding in the understanding of the role of IL-15 in the pathogenesis of CTCL (97, 98). It helps identify potential therapeutic targets, including the regulation of Zeb1 and exploring inhibitors of HDAC and miR-214 (97, 99). The highlighted negative regulatory relationship between miR-29b and BRD4 opens up new avenues for preventing the progression of CTCL (100).

It is important to note that while the IL-15 overexpression mouse model provides valuable insights into the role of IL-15 in CTCL, it cannot explain the mechanisms by which IL-15 overexpression occurs in patients. Therefore, further studies are needed to understand this fully and develop effective treatments.

Based on the latest advances in CTCL research, we aim to create autochthonous mouse models that can effectively replicate the disease's initial skin-based progression (1). These models are designed to modify genes in skin-homing CD4+ T cells and local inflammation, closely mimicking the natural development of CTCL. They significantly enhance our understanding of the origins of early-stage CTCL and show promise in designing early intervention measures.

The scope of this thesis

This thesis starts with an overview in **Chapter 1**, introducing the application of *in vivo* mouse models in the study of CTCL. These mouse models are crucial for deciphering the pathogenesis of the disease and testing potential treatment methods. My Ph.D. pursuit aims to establish Skin-Targeted Genetically Engineered Mouse Models (GEMMs) using gene alterations found in patient tumors through high-throughput sequencing, which might be the key factors to trigger CTCL. These models are intended to investigate the pathogenic role of these genes in the early stages of CTCL. Insights from these works are expected to contribute to the advancement of CTCL research and personalized treatment strategies.

In **Chapter 2**, we first obtained GEMM mice capable of specific *Socs1* knockout in skin-homing CD4+ T cells through breeding. We explored optimal conditions for tamoxifen administration outside the skin. Subsequently, an eight-week experiment using GEMM mice demonstrated that a single copy loss of *Socs1*, combined with persistent inflammation, was insufficient to initiate an early-stage mycosis fungoides-like phenotype within these mice in eight weeks.

To further confirm the causal role of *Socs1* allele loss in the development of MF, **Chapter 3** involves a larger group size (8-9) for stronger statistical power and extends the duration of the experiment. The experimentally induced contact allergic reaction continued for 20 weeks. Ten weeks after stopping the contact allergic challenge, we found that local *Socs1* mono-allelic loss in CD4+ T cells in chronically inflamed skin leads to autonomous skin inflammation with early MF characteristics.

Chapter 4 introduces a novel conditional knockout mouse model with *Hnrnpk* mono-allelic deletion in CD4+ T cells in the skin. Repeated contact allergic challenges were performed to maintain prolonged skin inflammation for 20 weeks, followed by a 20-week period without further treatment. This model mimics key features of early CTCL, including chronic skin inflammation, CD3+ CD4+ cell infiltration, and minimal disturbance in peripheral blood. It offers an experimental pathway to study complex microenvironments and immune

responses, enabling in-depth research into the function of *HNRNPK*, especially regarding de novo development against CTCL.

In **Chapter 5**, we utilized the novel strain of homozygous and heterozygous mice developed in **Chapter 4** to elucidate the role of *Hnrnpk* as an initiating factor when deleted in skin-homing CD4+ T cells, combined with repeated exposure to OXA. We further investigated the role of *Hnrnpk* deletion as an initiating factor in the pathogenesis of CTCL in skin-resident CD4+ T cells.

Chapter 6 presents a comprehensive overview of the data gathered in this study, along with an exploration of both clinical and research implications associated with this thesis.

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